

CANADIAN CENTRE FOR DNA BARCODING

DNA Testing Laboratory Report

Date of issue: May 22, 2013

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FORENSIC CASE INFORMATION

File Number: BIO_WCPO-TV9_2013-05-01
Client Name: Jason Law, Investigative Reporter
Client Address: 1720 Gilbert Avenue
Cincinnati, Ohio, USA
45202
Tel: (904) 377-0933
Contact Name: Jason Law (jason.law@wcpo.ca)

ITEMS

Description: 15 fish samples from area restaurants and grocery stores
Dates Received: May 01, 2013
Received From: Sue-Ann Connolly
Information Coordinator, Canadian Centre for DNA Barcoding
c/o United States Postal Service
Dates of Analysis: May 01 – May 16, 2013
Collector/Collection Site: Chris Weland / Canadian Centre for DNA Barcoding, Biodiversity of Ontario,
University of Guelph, 507 Gordon Street, Guelph

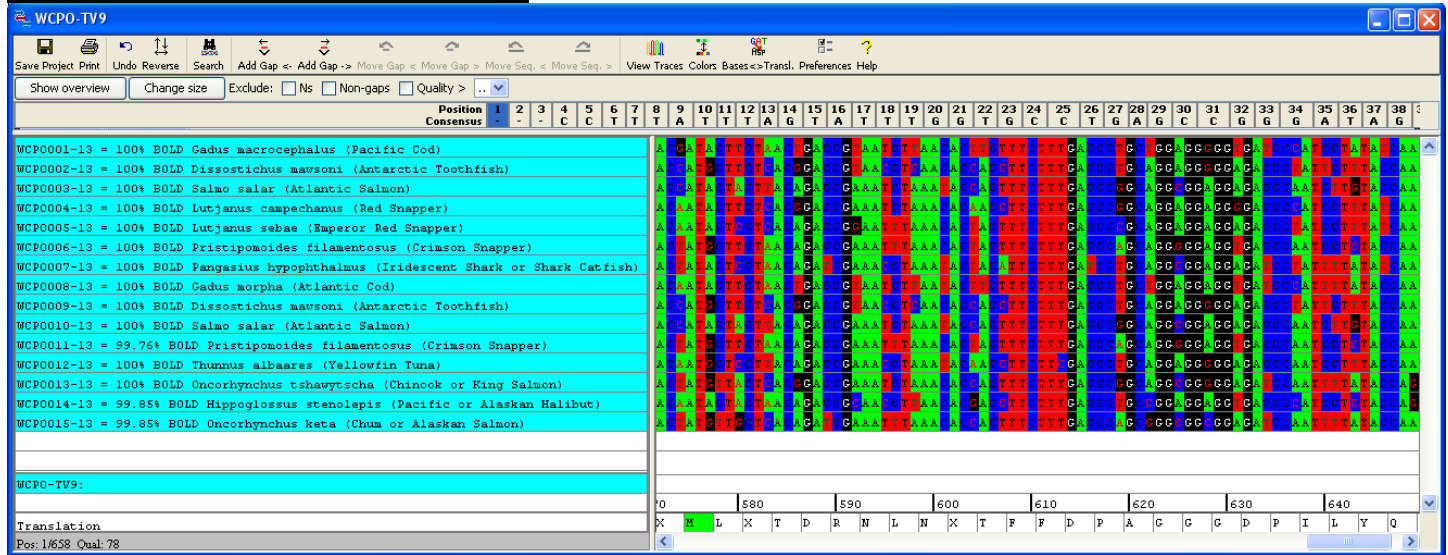
METHOD

To ascertain the identity of the species, DNA from each sample was extracted by organic DNA extraction protocol. Using universal primer cocktails *AquaF2* + *C_FishR1t1*, *RonM1_t1* + *C_FishR1_t1*, and *C_FishF1t1* + *C_FishR1t1*, the target genetic marker (barcode region of the mitochondrial DNA) was amplified using the Polymerase Chain Reaction (PCR), followed by cycle sequencing with a standardized commercially available BigDye Terminator v3.1 kit and subsequently detected by high-voltage capillary electrophoresis on an automated ABI 3730xL DNA Analyzer. DNA sequences recovered from the unknown samples were compared against the species sequence reference library in the Barcode of Life Data System (BOLD) accessible at www.boldsystems.org.

IMAGING

All items were photographed in the Forensic Item Examination Area by Andrew Young, Laboratory Assistant - Collections of Specimen Imaging for the CCDB, using a Canon EOS 50D digital SLR camera. Pictures were uploaded to the BOLD website into the secure project called "WCPO-TV9 Fish Market Survey [WCPO]". Please refer to the BOLD project for a full image inventory from each product. Also see Appendix 1 for select sample images.

DNA SEQUENCING RESULTS



DNA SPECIES IDENTIFICATION MATCH PERCENTAGE

BOLDSYSTEMS		Databases	Taxonomy	Identification	Workbench	Resources
Specimen Identification Request						Print
Query: WCPO001-13 = Cod Loins, clerk "Alaskan" or label "Wild Caught China"		Top Hit: Chordata - Gadiformes - <i>Gadus macrocephalus</i> (100%)				
Query: WCPO002-13 = Chilean Sea Bass		Top Hit: Chordata - Perciformes - <i>Dissostichus mawsoni</i> (100%)				
Query: WCPO003-13 = Atlantic Salmon, Chile Farm Raised		Top Hit: Chordata - Salmoniformes - <i>Salmo salar</i> (100%)				
Query: WCPO004-13 = Wild American Red Snapper		Top Hit: Chordata - Perciformes - <i>Lutjanus campechanus</i> (100%)				
Query: WCPO005-13 = Snapper, clerk "Red" or label "Wild Caught in Indonesia"		Top Hit: Chordata - Perciformes - <i>Lutjanus sebae</i> (100%)				
Query: WCPO006-13 = Grilled Snapper, server "Red"		Top Hit: Chordata - Perciformes - <i>Pristipomoides filamentosus</i> (100%)				
Query: WCPO007-13 = Grilled Snapper, server "Red"		Top Hit: Chordata - Siluriformes - <i>Pangasius hypophthalmus</i> (100%)				
Query: WCPO008-13 = Pacific Cod		Top Hit: Chordata - Gadiformes - <i>Gadus morhua</i> (100%)				
Query: WCPO009-13 = Chilean Sea Bass		Top Hit: Chordata - Perciformes - <i>Dissostichus mawsoni</i> (100%)				
Query: WCPO010-13 = Salmon, server "Atlantic"		Top Hit: Chordata - Salmoniformes - <i>Salmo salar</i> (100%)				
Query: WCPO011-13 = Snapper Hemingway, server "snapper raised in the gulf"		Top Hit: Chordata - Perciformes - <i>Pristipomoides filamentosus</i> (99.76%)				
Query: WCPO012-13 = Yellow Fin Tuna		Top Hit: Chordata - Perciformes - <i>Thunnus albacares</i> (100%)				
Query: WCPO013-13 = King Salmon		Top Hit: Chordata - Salmoniformes - <i>Oncorhynchus tshawytscha</i> (100%)				
Query: WCPO014-13 = Alaskan Halibut		Top Hit: Chordata - Pleuronectiformes - <i>Hippoglossus stenolepis</i> (99.85%)				
Query: WCPO015-13 = Wild Alaskan Salmon		Top Hit: Chordata - Salmoniformes - <i>Oncorhynchus keta</i> (99.85%)				

INTERPRETATION

Bidirectional forward and reverse sequences were generated for each sample. Resulting trace files were assembled into contigs and consensus sequences were then manually edited in CodonCode Aligner (version 4.1.1.) software. Sequences of the mitochondrial DNA COI gene were compared against the reference sequence records available in BOLD, the Barcode of Life Data Systems. Based on a percentage of nucleotide sequence divergence (number of nucleotide substitutions) between a sequence from test sample and a reference DNA barcode, the closest match was used to infer species identity of the DNA contributor in the corresponding test sample. Images, primers, sequences and their associated trace files with quality scores were uploaded to the secure BOLD project called "WCPO-TV9 Fish Market Survey [WCPO]".

SUMMARY

DNA Barcodes were generated from 15 of 15 fish samples from area restaurants and grocery stores. All fifteen (15) were identified as samples with DNA source from a single species. Two (2) samples were misidentified. Sample#7, from Washington Platform – 1000 Elm street, identified as Grilled Snapper (server said "Red") was actually *Pangasius hypophthalmus* the Iridescent Shark, a species of shark catfish. Sample#8, from Mitchell's – 1 Levee Way #2129, Newport, identified as Pacific Cod was actually *Gadus morhua*, the Atlantic Cod.

In addition, samples # 2 and # 9, from Fresh Market – 7888 Montgomery Road and Bonefish Grill – 2737 Madison Road, both identified as Chilean Sea Bass were actually *Dissostichus mawsoni*, the Antarctic toothfish. Dr. Tyler Zemplak, Director of Business Development for the CCDB and expert in Fisheries Identification, comments "the Chilean Sea Bass is also known as Patagonian Toothfish (*Dissostichus eleginoides*) and closely related to the Antarctic Toothfish (*Dissostichus mawsoni*). These two species have overlapping distributions and could be a simple clerical error by the fisherman." Dr. Dirk Steinke, Director, Education and Outreach for BIO and expert in Fisheries Identification, further comments "Indeed that might be a clerical error that actually reflects where they are mainly fishing (most fleets for toothfish are around Antarctica) but if you are strictly going by the book (here FDA list) it is wrongly labeled".

CONCLUSIONS

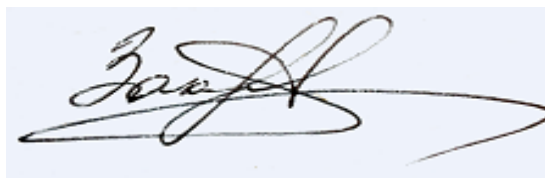
The present testing has provided species-level identifications of 15 of 15 fish samples from Ohio area restaurants and grocery stores submitted by Jason Law, Investigative Reporter, WCPO-TV channel 9 News. Based on appropriate statistical BOLD match calculations and a reasonable degree of scientific certainty of the BOLD reference library, species identity of detected DNA source in all fish samples is considered practically proven.

RESULTS REPORTED BY:

A handwritten signature in blue ink, appearing to read 'C. Weland', with a large, stylized 'C' at the beginning.

Chris D. Weland, Forensic Scientist, CCDB

RESULTS REVIEWED BY:

A handwritten signature in black ink, appearing to read 'Evgeny V. Zakharov', with a large, stylized 'E' at the beginning.

Dr. Evgeny V. Zakharov, Director, CCDB

All inquiries pertaining to this report should be directed to C. Weland (cweland@uoguelph.ca).
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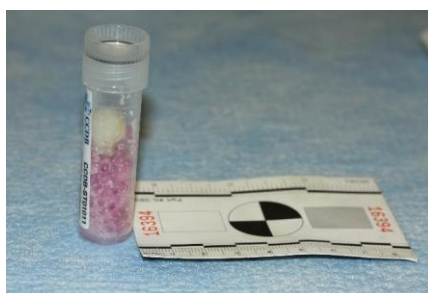
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Appendix 1. Sample Images

CCDB-ST01011, REMKE-6920 Burlington Pike, Florence

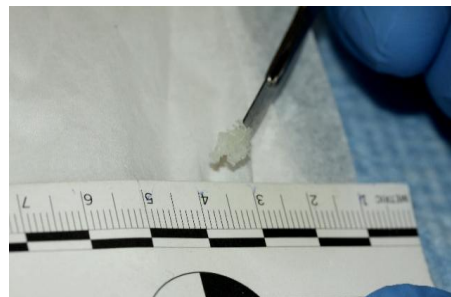


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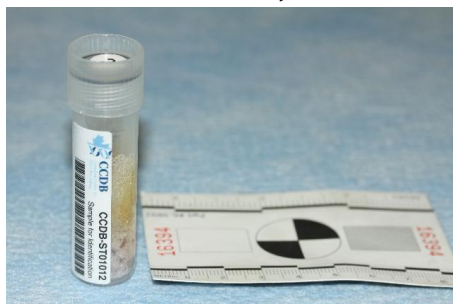
Back

WCPO001-13

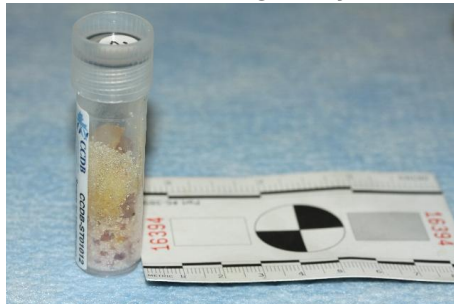


Fish Subsample

CCDB-ST01012, FRESH MARKET-7888 Montgomery Rd



Front



Back

WCPO002-13

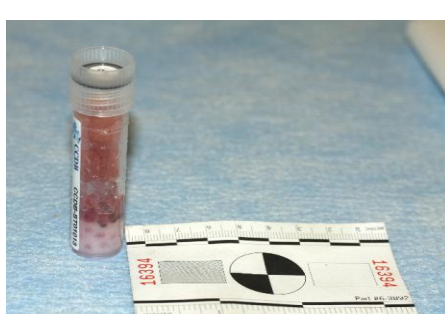


Fish Subsample

CCDB-ST01013, MEIJER-6550 Harrison Ave



Front



Back

WCPO003-13

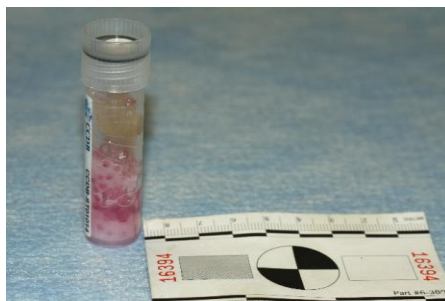


Fish Subsample

CCDB-ST01014, WHOLE FOODS-2693 Edmonston Rd

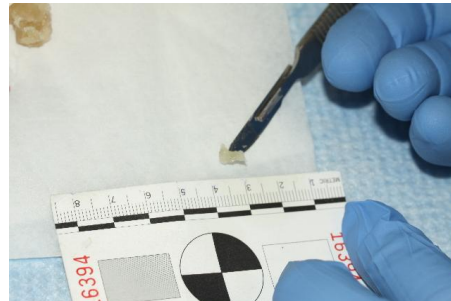


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Back

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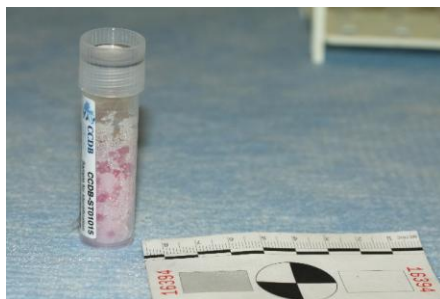


Fish Subsample

CCDB-ST01015, KROGER-5830 Harrison Ave

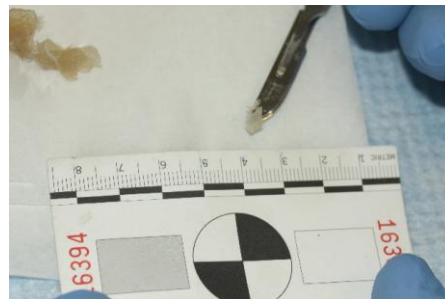


Front



Back

WCPO005-13



Fish Subsample

CCDB-ST01016, JOE'S Crab Shack-25 Fairfield Ave, Bellevue

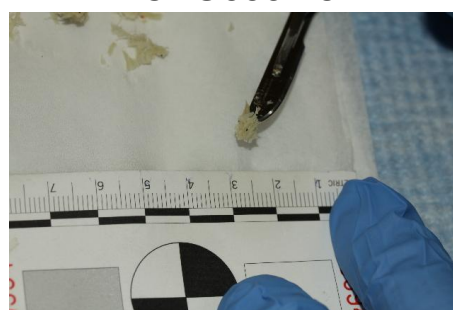


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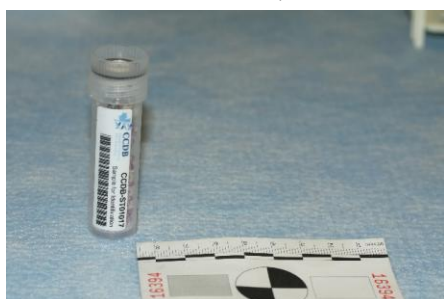
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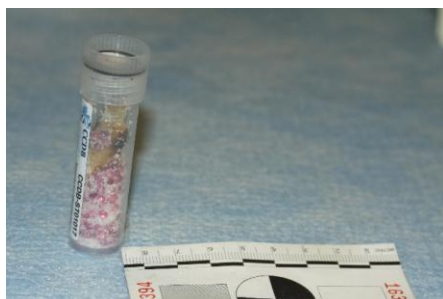


Fish Subsample

CCDB-ST01017, WASHINGTON PLATFORM-100 Elm Street



Front



Back

WCPO007-13

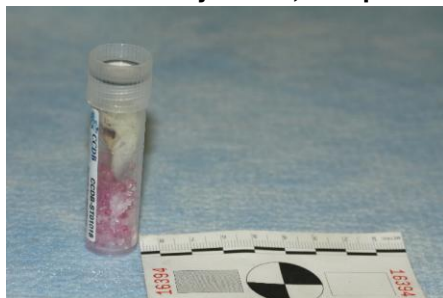


Fish Subsample

CCDB-ST01018, MITCHELL'S-1 Levee Way #2129, Newport

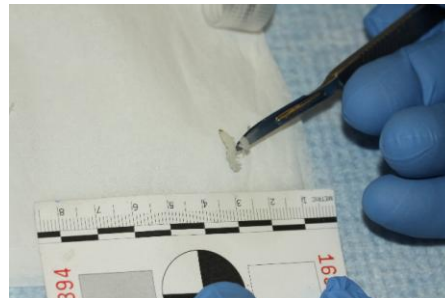


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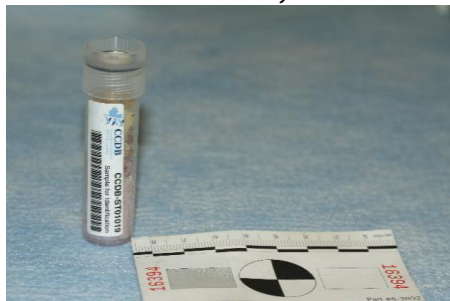
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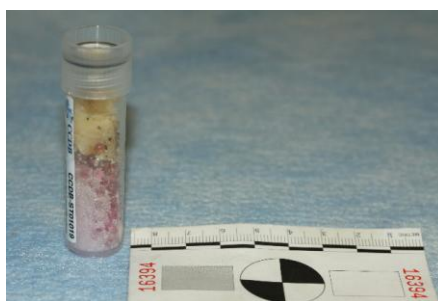


Fish Subsample

CCDB-ST01019, BONEFISH GRILL-2737 Madison Rd

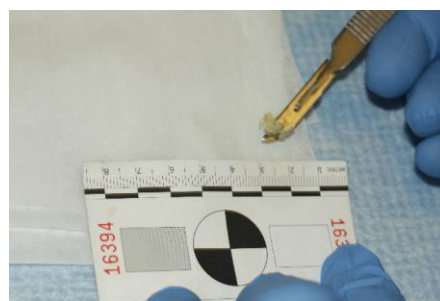


Front



Back

WCPO009-13



Fish Subsample

CCDB-ST01020, RED LOBSTER-8220 Montgomery Rd



Front



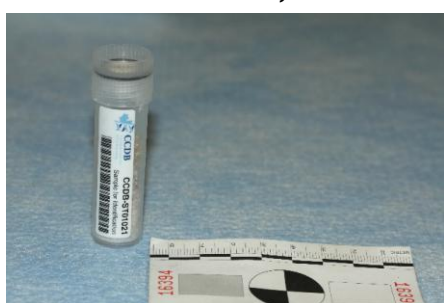
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WCPO010-13



Fish Subsample

CCDB-ST01021, CHART HOUSE-405 Riverboat Row, Newport

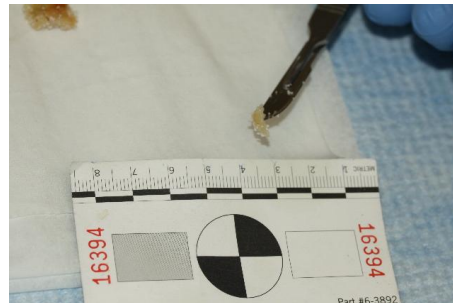


Front



Back

WCPO011-13

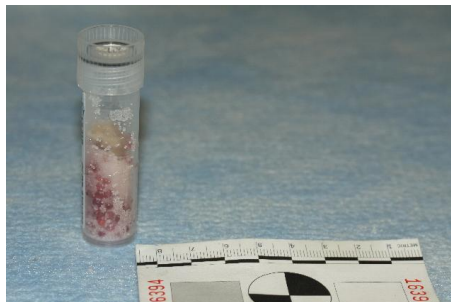


Fish Subsample

CCDB-ST01022, JEFF RUBY'S 'THE PRECINCT'-311 Delta Ave WCPO012-13



Front

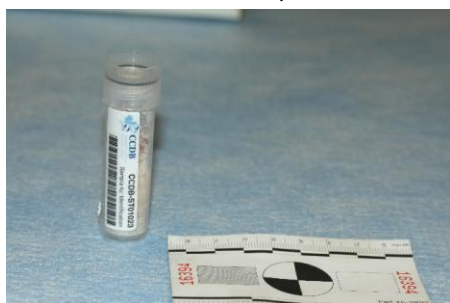


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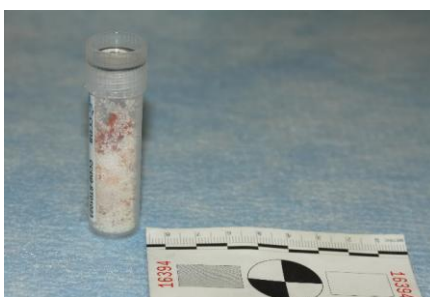


Fish Subsample

CCDB-ST01023, McCORMICK & SCHMICK'S-21 E 5th St



Front



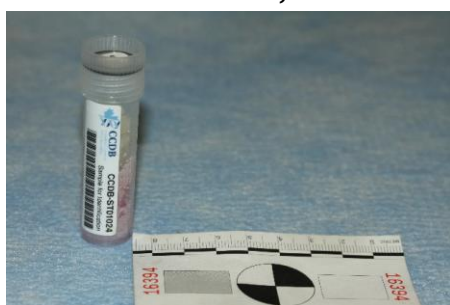
Back

WCPO013-13

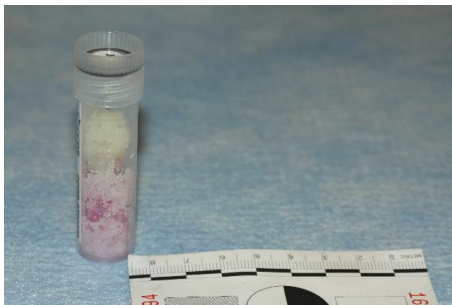


Fish Subsample

CCDB-ST01024, THE ANCHOR-1401 Race Street



Front



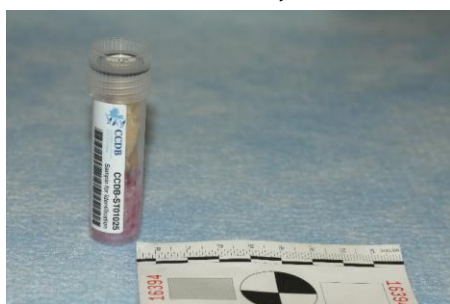
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WCPO014-13

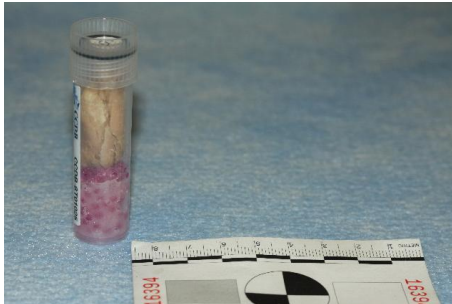


Fish Subsample

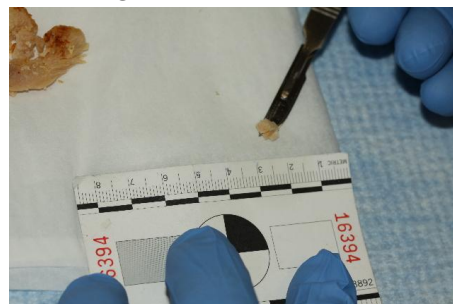
CCDB-ST01025, CAPTAIN D'S SEAFOOD KITCHEN-7000 Reading Rd WCPO015-13



Front



Back



Fish Subsample